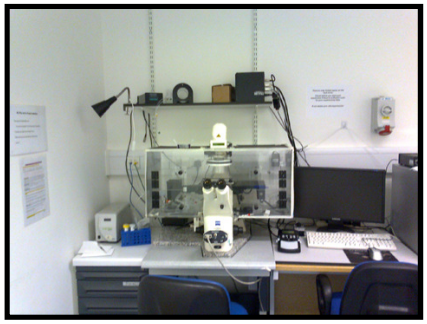
OPERATING INSTRUCTIONS



TIMELAPSE inverted epi-fluorescence microscope Advanced

You must not operate this equipment without prior training from a BALM facility staff member.

To arrange training and for help please contact:

Dr Ann Wheeler	ext: 2406	a.p.wheeler@qmul.ac.uk
Dr Katy Cogger	ext: 2407	k.f.cogger@qmul.ac.uk
Isma Ali	ext: 2407	i.ali@qmul.ac.uk

Standard Operating Procedure — Advanced user guide

How to turn the equipment on:

1. If you want to do fluorescence, switch on the metal halide lamp (a)
2. Switch on the extension plug (b)
3. **NEW: Switch on the Camera – using the button on the camera (It looks like a computer on button)**
4. Switch on the computer and log in
5. If you want to do bright field, switch on the bright field controller box (d)

How to turn the equipment off:

Switch off the bright field controller box, then the computer, then extension plug (b), and finally the metal halide lamp (a)

Rules of use:

This microscope should be treated with respect and care at all times.

This Microscope can only be used by Masters by Research or PhD students, Postdocs and members of staff.

The microscope lenses must be cleaned after every usage and the equipment treated carefully at all times.

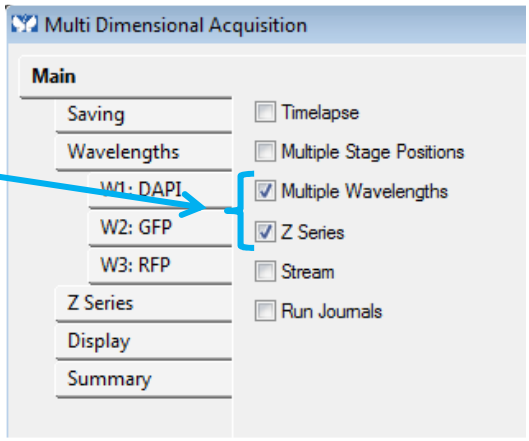
If you have any problems at all with the microscope, no matter how trivial they may seem please see a technician immediately.

REMEMBER: You have 20GB of disk space on this microscope. Check before you start if you have room for your experiment. If not, delete your old data. Make sure you empty the recycle bin afterwards

Z-stacks

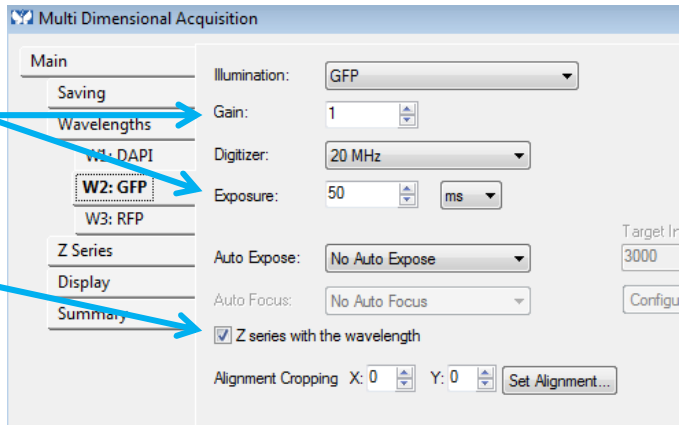
1) In Multi Dimensional Acquisition select **Multiple Wavelengths** and **Z series**

2) Save as normal
(files are saved as a single file for each wavelength)



3) Set gain and exposure for each wavelength for the brightest point in the Z axis (live)

4) Make sure **Z series with the wavelength** is ticked for each channel

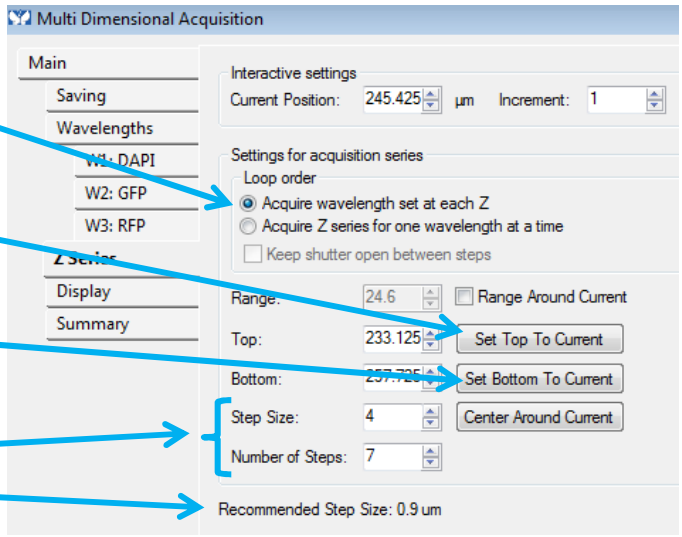


5) In the **Z series** tab (live)
Either – Acquire wavelength set at each Z
Or – Acquire Z series for one wavelength at a time

6) Focus on top/bottom of image and click **Set top to current**

7) Focus in opposite direction and click **Set bottom to current**

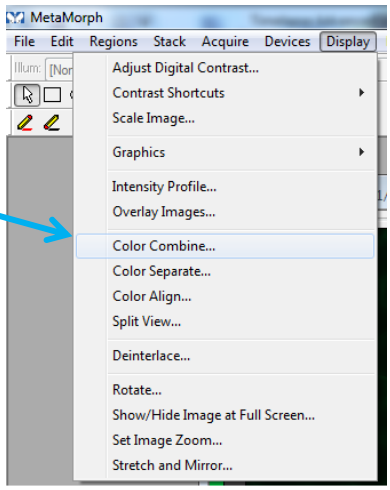
8) Choose step size or number of steps
(see recommended step size for guidance)



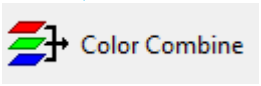
9) Click **Acquire**

Processing Z-stack data

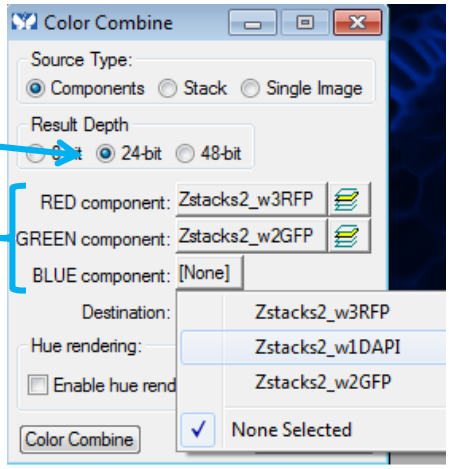
To create a multi-coloured stack, select **Display**, and **Colour Combine**,



or use the **Colour combine** shortcut



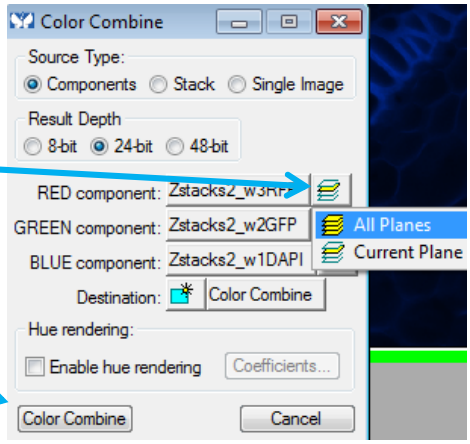
Set the bit depth to **24-bit**, and select the stacks that correspond to each colour component



Make sure **All Planes** of the stack are selected by clicking on the stack icon

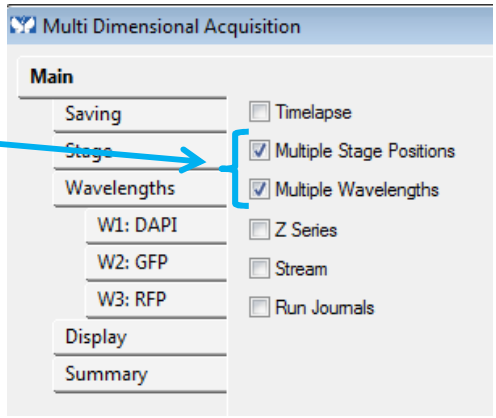
Click **Colour Combine**

Remember to save the colour combined stack



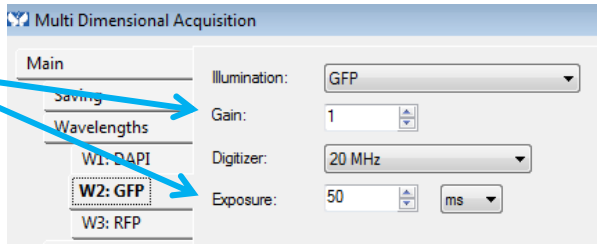
Multiple stage positions

1) In Multi Dimensional Acquisition select **Multiple Stage Positions** and **Multiple Wavelengths**



2) Save as normal
(files are saved as a separate file for each wavelength at each position)

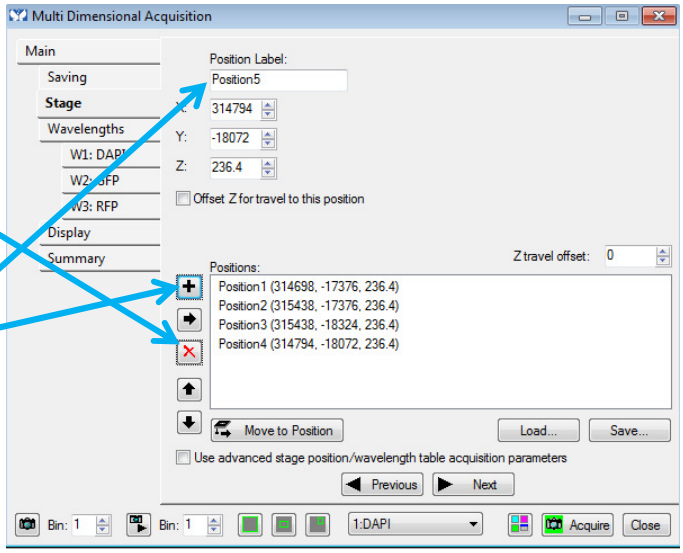
3) Set gain and exposure for each wavelength at one of the positions (live)



4) In the **Stage** tab (live),
Remove any stage positions already stored from previous experiments by highlighting them and selecting **Delete**

5) Using the joystick move the stage to a position of interest, focus, type in a label for the position, then click **Add current position to list**

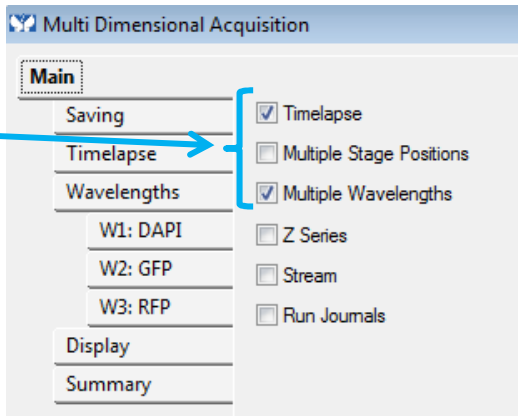
6) Move to the next position of interest and add it to the list, and so on



7) Click **Acquire**

Timelapse

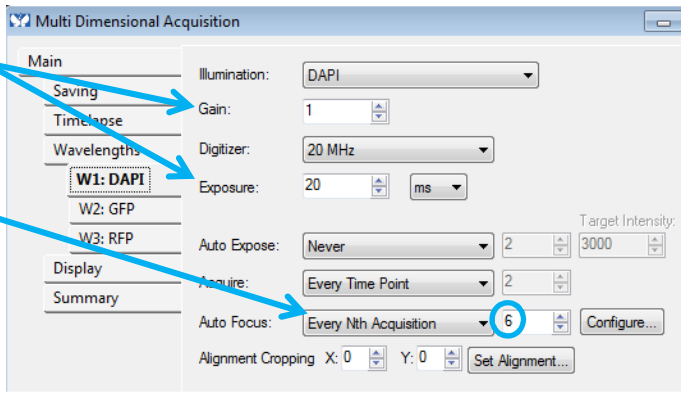
1) In Multi Dimensional Acquisition select **Timelapse**, and **Multiple Wavelengths** if you are using more than one channel



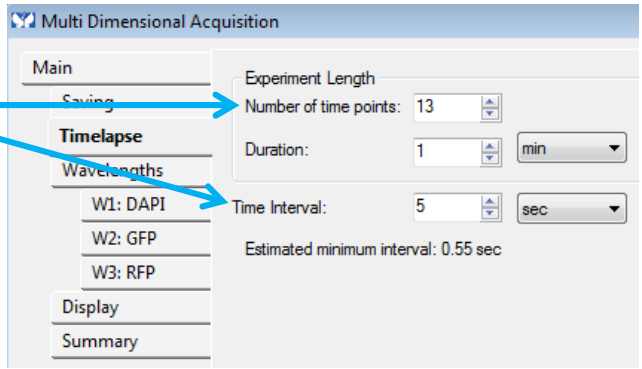
2) Save as normal
(files are saved as a single file for each wavelength at each time point)

3) Set gain and exposure of each wavelength (live)

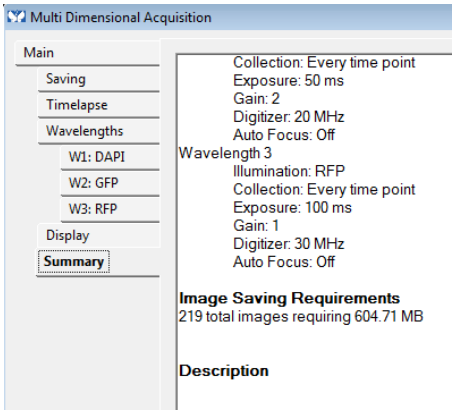
4) Make sure Auto Focus is set for **Every Nth Acquisition** and N is set to **6**



5) In the **Timelapse** tab,
Set the **Number of time points** and **Time Interval**
Duration tells you how long the experiment will take



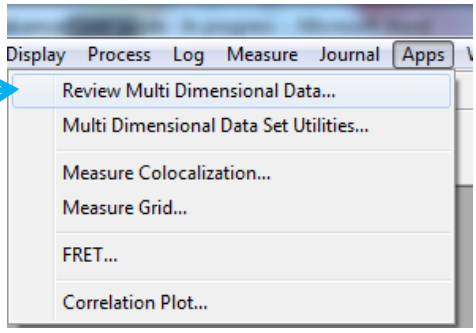
6) In the **Summary** tab, you can see how much storage space is required to collect you data set. Make sure you have enough space on the E-Drive, and empty the recycle bin.



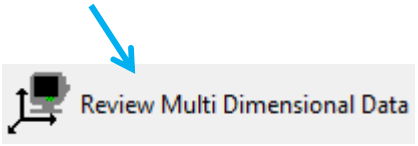
7) Click **Acquire**

Processing timelapse data

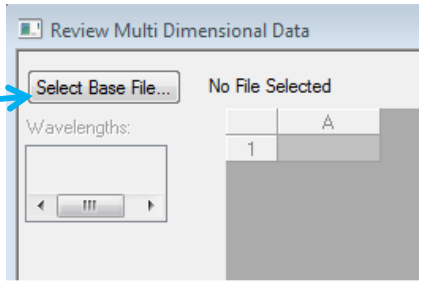
To make a metamorph timelapse movie, go to **Apps** and **Review Multi Dimensional Data**



Or use the **Review Multi Dimensional Data** shortcut



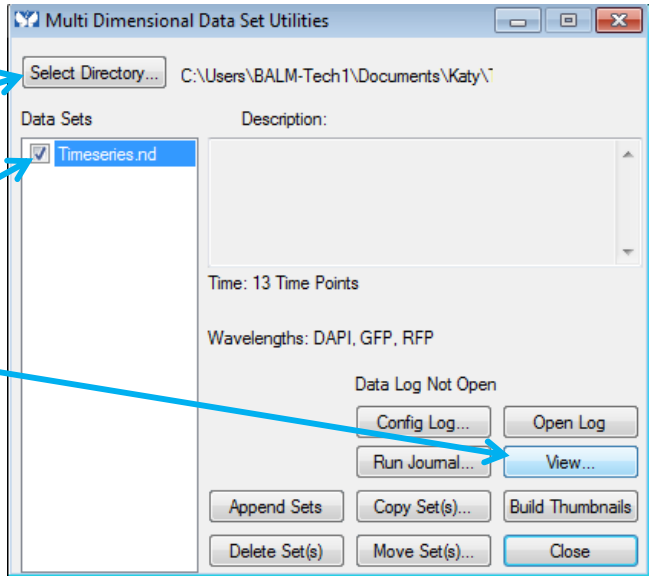
In the **Review Multi Dimensional Data** window click **Select Base File**



In the **Multi dimensional data set utilities** window click **Select Directory** and find where you saved your data

Make sure you tick the dataset you want to use

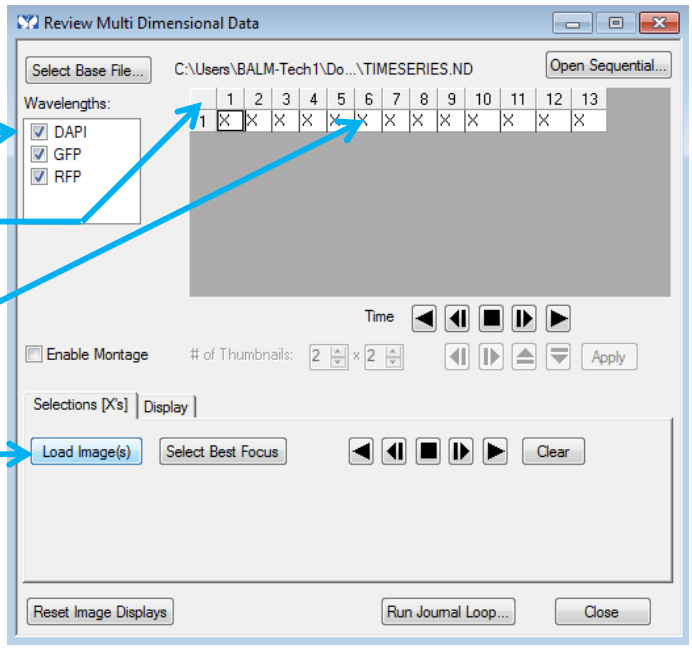
Then click **View**



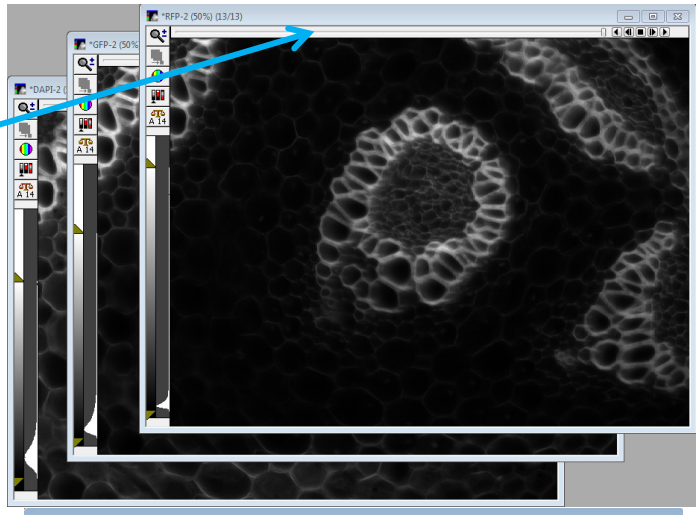
In the **Review Multi dimensional Data** window tick the wavelengths you want to use

Right click in the top left corner to select all time points
NB. If you do not want to include all time points you can unselect them here by right clicking in the box for each image

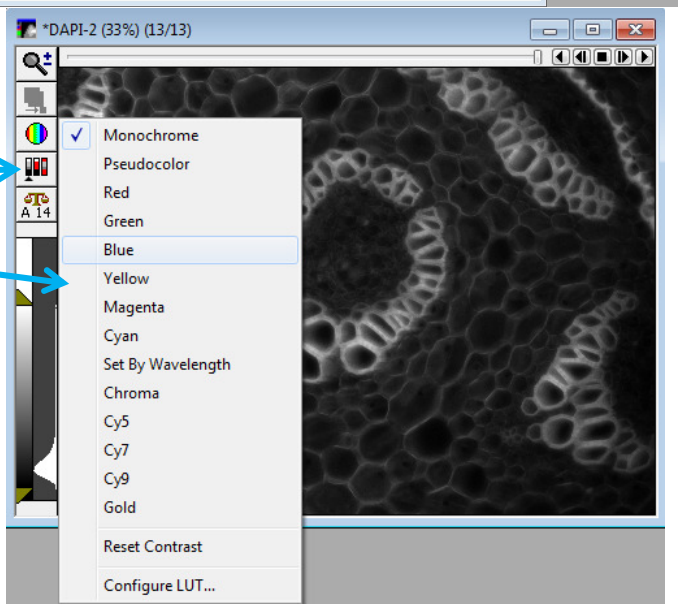
Click **Load Images**



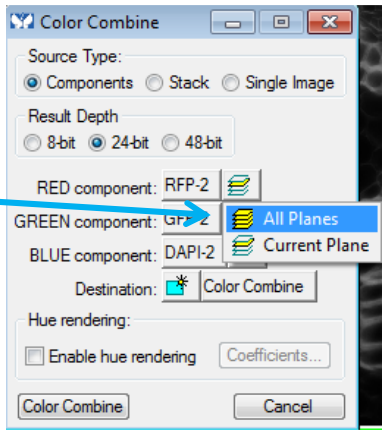
A separate stack/movie is created for each wavelength, use the bar at the top to navigate through the different time points



You can change the colour of the stack by clicking on the look up tables button, and selecting a colour

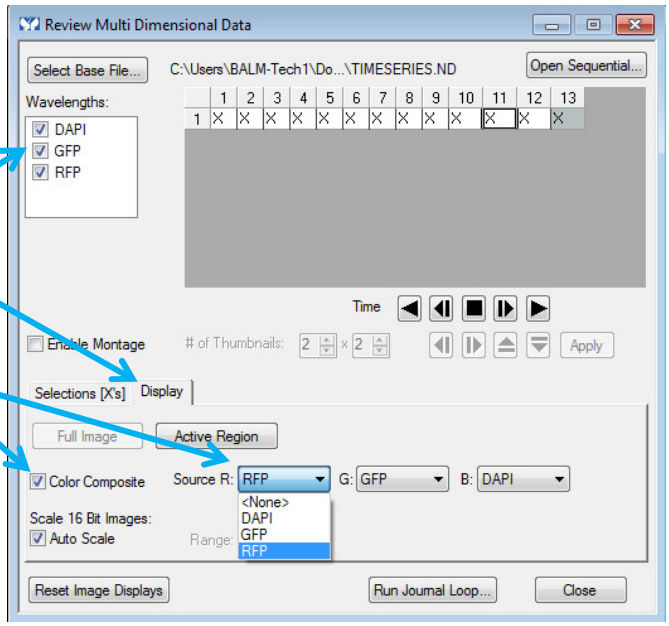


To combine different coloured stacks, use the **Colour Combine** tool (Display – Colour combine), and make sure **All Planes** are selected (See processing Z-stack data for more info)

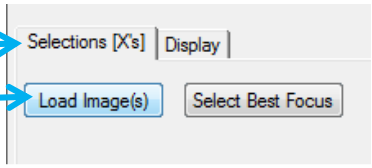


Alternatively, you can make a multi-coloured stack/movie using **Review Multi Dimensional Data**.

Select the wavelengths you want to combine, then in the **Display** tab, tick the **Colour composite** box, and select the correct sources for each wavelength



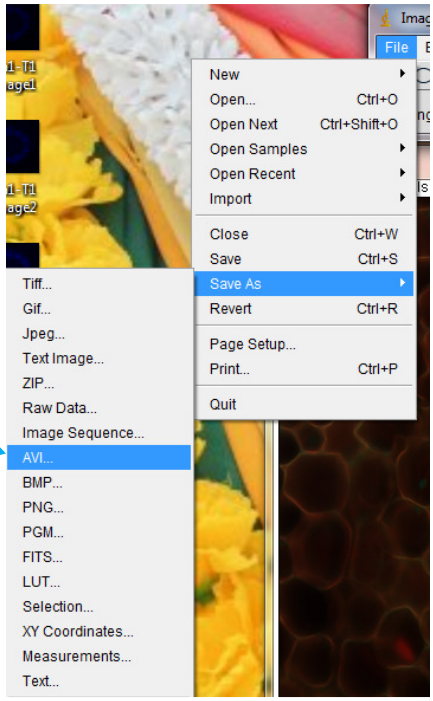
Go back to the **Selection** tab and click **Load images** as before



Remember to save your movie/stack (usually in Tif format)

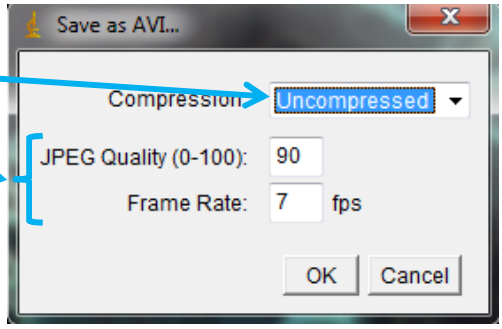
To change your metamorph movie/stack into a universally compatible format, open the Tif file in **Image J**

Then go to **File, Save As**, **AVI**



Make sure you choose **Uncompressed**

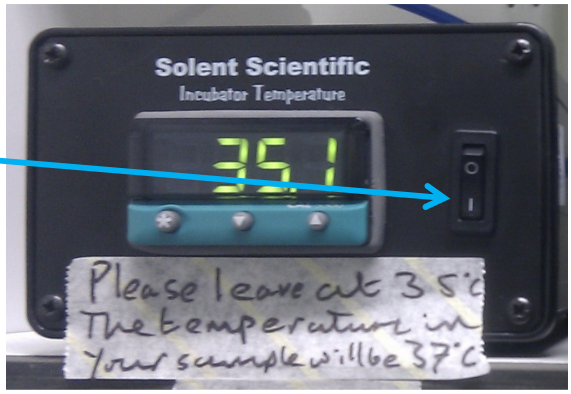
And then decide the **Quality** and **Frame Rate**



Live cell imaging – switching on the system

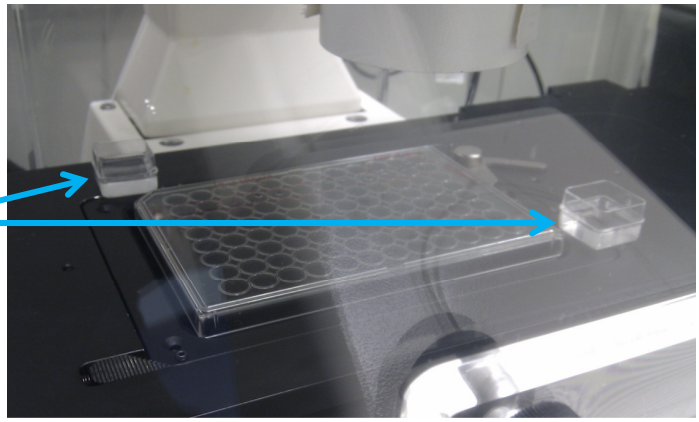
1) Switch on the temperature controller
   NB. System takes about 1hr to warm up

   Please leave the controller set to 35°C, the temperature at the sample will be 37°C

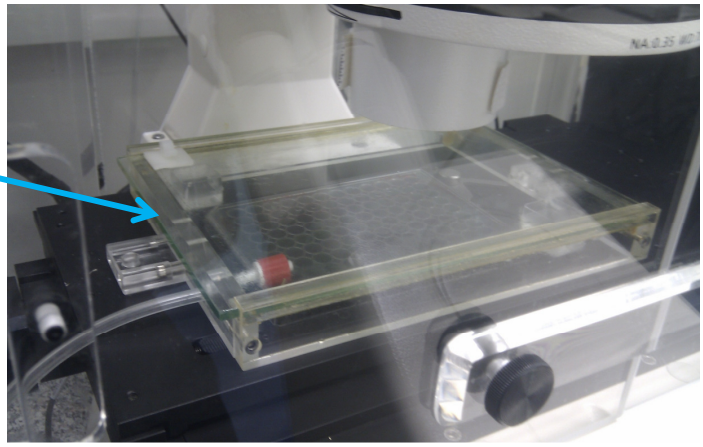


2) Put sample on stage

3) Position water chambers around sample

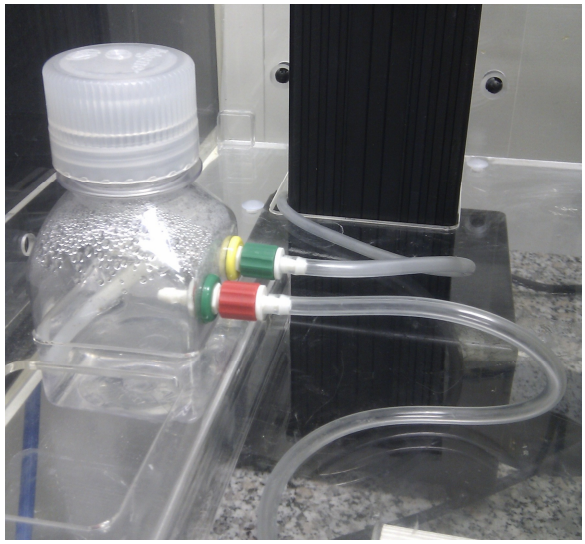


4) Position CO_2 chamber over sample ensuring it is sitting flat, and there are no gaps

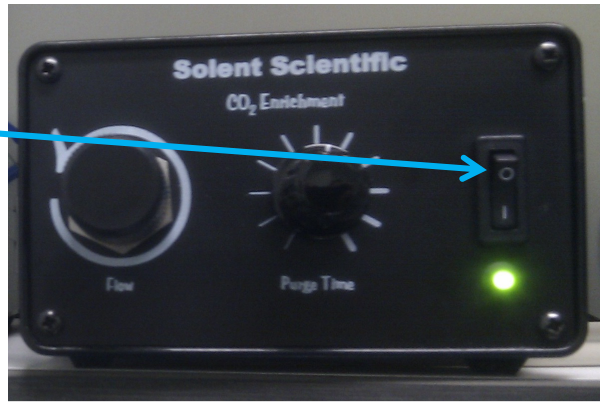


5) Check tubing is connected to the ddH_2O bottle

NB. Only one bottle should be connected

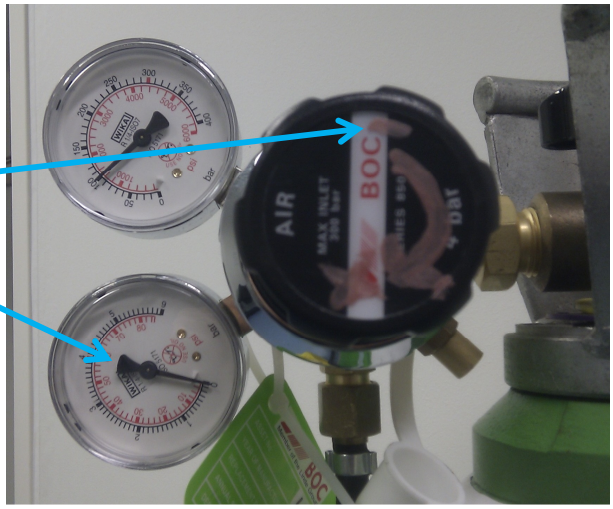


6) Turn on CO_2 controller

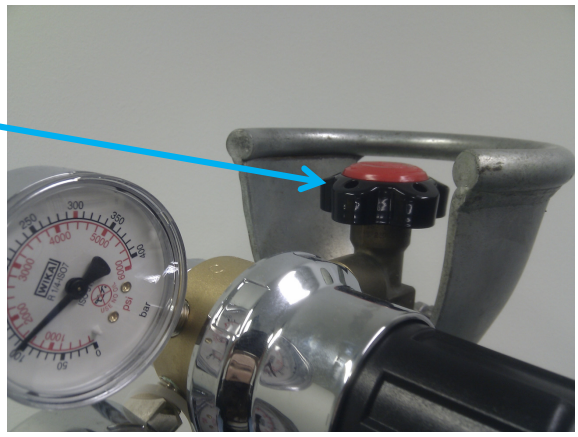


7) Check there is no pressure left in the system by turning the side valve on the gas cylinder in the minus direction (ie. Anti-clockwise). The pressure on the bottom gauge should read zero.

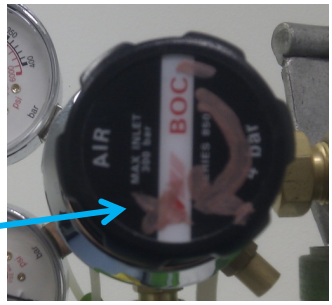
If you do not check this, the tubing can EXPLODE when you open the CO_2 air valve



8) Open the CO_2 air valve on the top of the gas cylinder



9) Adjust the side valve (clockwise) to increase the pressure in the system to 1 bar (bottom gauge)

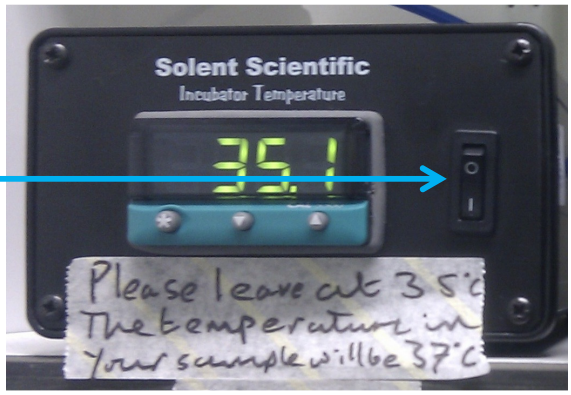


10) Check you can see bubbles in the water bottle

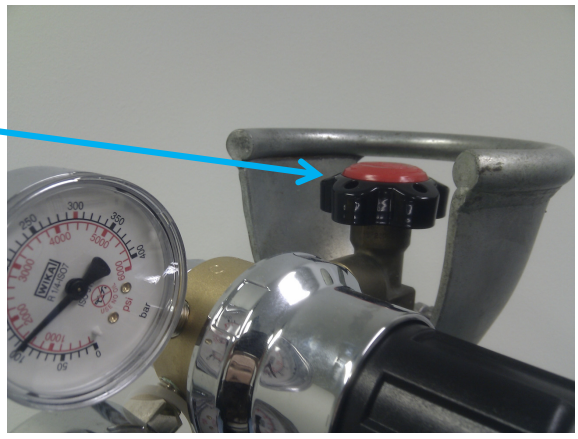
11) Set up Metamorph

Live cell imaging – switching off the system

1) Turn off the temperature controller

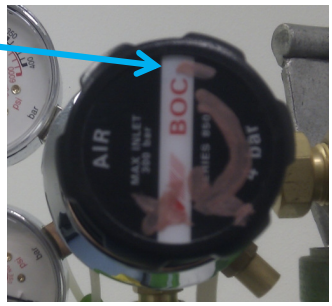


2) Close the top valve on the gas cylinder



3) Turn the side valve in the minus direction (anti-clockwise) to release the pressure in the system

4) Wait for the gauge to return to zero, and the bubbles in the water bottle to stop



5) Turn off the CO_2 controller